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SHORT COMMUNICATION

Annual cycles of bacterioplankton biomass and production suggest a general switch between temperature and resource control in temperate coastal ecosystems

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Factors regulating the biomass and flux of organic carbon through heterotrophic bacterioplankton were examined monthly in the southern Bay of Biscay during 2006 and 2007. Temperature and resource supply were able to explain significantly changes in bacterial biomass (539–1389 mg C m^{-2}) and production (5.4–93 mg C m^{-2} day^{-1}), although at different periods of the year. A switch between the relative strength of both factors in the spring–summer transition seems to be a general feature in temperate coastal waters.

KEYWORDS: bacterial biomass; bacterial production; bottom-up; temperature control; continental shelf waters

Given the importance of heterotrophic bacterioplankton in marine biogeochemistry (Fuhrman, 1992), factors regulating the biomass and productivity of bacterial communities and the magnitude of bacterial carbon flux are

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central topics in marine microbial ecology. Control by resource supply (bottom-up) and by mortality (top-down), either bacterivory or viral lysis, along with temperature are the main factors affecting bacterial biomass and production (Ducklow and Carlson, 1992). Their relative importance may vary with the time or space scale considered (Ducklow, 1992), but these controlling processes frequently act simultaneously on bacteria, making it difficult to isolate a single key factor. Although substrate supply has long been recognized as the major factor regulating biomass and growth rates of bacterial communities in aquatic ecosystems (Church, 2008), some studies have suggested that temperature can play a predominant role in meso- to eutrophic ecosystems, at least during certain seasons (Billen *et al.*, 1990), and their relative importance might change seasonally (Shiah *et al.*, 2003). A geographical component related to the trophic state of the system has also been shown by Shiah *et al.* (Shiah *et al.*, 1999), according to which seasonal variations in eutrophic waters (coastal and estuarine systems) might be primarily regulated by temperature, whereas resource supply could be the main controlling factor in open ocean oligotrophic waters.

After describing the temporal patterns of both biomass (BB) and production (BP) of heterotrophic bacteria at three stations in the central Cantabrian Sea (southern Bay of Biscay) over a whole year, the main purpose of this study is to identify the role of temperature and substrate supply in controlling these patterns. Relationships with temperature and the analysis of regression models between BB and BP were used (Ducklow, 1992) over two complete annual cycles (January 2006–January 2008).

Monthly sampling was carried out during 2006 on board of RV "José de Rioja" at three stations (St 1: 43.58°N, 5.61°W; St 2: 43.67°N, 5.58°W; St 3: 43.78°N, 5.55°W) routinely sampled within the RADIALES time-series program off Xixón, on the southern Bay of Biscay continental shelf (Calvo-Díaz and Morán, 2006). Water samples were collected in 5-L Niskin bottles and processed as detailed elsewhere (Morán and Calvo-Díaz, 2009). Samples were taken from different depths from the surface down to the near bottom for BB (from 4 to 9 depths) and for BP (from 3 to 5 depths). Maximum depths were 20 (St 1), 100 (St 2) and 150 m (St 3). Chlorophyll *a* (chl *a*) concentration was measured fluorometrically in acetone extracts. Phytoplankton biomass (phytoB) was estimated from chl *a* measurements assuming a constant C:chl *a* ratio of 50. Total bacterial counts were estimated from samples preserved with 1% paraformaldehyde + 0.05% glutaraldehyde and frozen at −80°C until analysis in the laboratory with an FACSCalibur flow cytometer (Becton-Dickinson). Details of sample processing and estimation of bacterial abundance can be found in Morán and Calvo-Díaz (Morán and Calvo-Díaz, 2009).

Monthly temperature data, BB and BP collected at St 2 from February 2007 to January 2008 were added for the analysis of controlling factors on bacterial community. A detailed description of environmental and biological conditions during 2007 in the study area can be found in Franco-Vidal and Morán (Franco-Vidal and Morán, 2011).

Estimates of BB and BP both require assuming or empirically determining factors for converting abundance and radiotracer uptake rates, respectively, into carbon units. Here, we have minimized this source of error by using an empirical calibration between SSC and cell diameter (Calvo-Díaz and Morán, 2006) to estimate biovolume which was converted into bacterial carbon biomass by using the allometric relationship of Norland (Norland, 1993): $\mathrm{BB\ (pg\ C\ cell^{-1})} = 0.12(\mathrm{biovolume})^{0.72}$.

Leucine incorporation rate (LIR) was determined using the [^{3}H]leucine (Leu) incorporation method as detailed in Calvo-Díaz and Morán (Calvo-Díaz and Morán, 2009). Incubations were carried out inside photosynthesis-irradiance incubators with neutral density filters that simulated the irradiance at different depths. A comparison between light and dark incubations was carried out for surface (5 m depth, St 1, St 2 and St 3) and 50 m depth (St 2 and St 3) samples (data not shown). Light incubations of surface samples resulted on average in 24 ± 6% higher LIR values compared with dark incubations (paired *t*-test, $P < 0.001$, $n = 36$), whereas no significant differences were found for samples from 50-m depth (paired *t*-test, $P = 0.19$, $n = 23$) (Calvo-Díaz and Morán, pers. comm.). All values of BP appearing in the text hereafter are from the light experiments. Differences between *in situ* and experimental temperatures averaged 0.36 ± 0.17°C (SE).

Empirical Leu-to-carbon CF determined at the surface of the three stations every 2 months were used to convert rates of Leu uptake into BP (details in Calvo-Díaz and Morán, 2009). Given the strongly coherent seasonal pattern of empirical CFs, linear extrapolations between consecutive determinations were used for months without empirical values. Due to strong stratification, two additional CF experiments with water from 50-depth were also performed at St 2 and St 3 in August, and used for 50- and 100-m depth. For 50- and 100-m depth in July and September, the mean between correlative surface and 50 m August CF values was used. Monthly empirical CF were also determined at the surface and at 50-m depth at St 2 during 2007 (see details in Franco-Vidal and Morán, 2011) and were used to estimate BP in that period. The daily change of irradiance on 24 h-integrated BP values was not considered.

As previously described (Calvo-Díaz and Morán, 2006) typical hydrographic conditions of temperate

coastal zones were found in the study area, with a well-mixed water column in autumn and winter and marked summer stratification. Vertical and temporal distributions of temperature (range: 11.6–22.1°C), salinity (range: 34.3–35.8) and nitrate concentrations (range: 0.1–11.5 μmol L^{-1}) during 2006 are shown in more detail elsewhere (Morán and Calvo-Díaz, 2009; Morán *et al.*, 2010). Temperature stratification in summer and the presence of low salinity waters in winter frequently induced shallow (<20 m) upper mixed layers. Nutrient limitation (defined as a NO_3 concentration <1 μmol L^{-1}) characterized the mixed layer during strong stratification at temperatures generally warmer than 16°C (Morán *et al.*, 2010). Chl *a* maxima were found close to the surface in March at St 3 (7.6 μg L^{-1}), whereas St 1 and St 2 showed highest values in June (3.7 and 3.4 μg L^{-1}, respectively).

Values of BB and BP observed in this study (Fig. 1) were within the range of values published for coastal waters in other southern Bay of Biscay sites (González *et al.*, 2003). Changes in biomass (Fig. 1A) were mostly driven by changes in bacterial abundance, since variations in mean cell diameter were relatively small (0.41–0.58 μm). Yet, the consideration of a fixed cell carbon content (e.g. 20 fg C as commonly used, Fukuda *et al.*, 1998) would have yielded slightly higher values, since the annual average for our study zone was 15.75 fg C $cell^{-1}$, but there were no changes in the seasonal pattern. Lower biomass values were found in winter at all depths and deep in the water column in late summer and early autumn (<10.2 μg C L^{-1}) with surface maxima in August at the three stations (22.2–37.6 μg C L^{-1}) and also in May at St 2 and St 3 (Fig. 1A). Water-column (0–100 m for St 2 and 0–150 m for St 3) integrated values of BB at the most offshore stations ranged from 539 to 1389 mg C m^{-2}, very close to those found at St 2 a year afterwards (557–1558 mg C m^{-2}, Franco-Vidal and Morán, 2011).

With the uncertainties associated with a fixed chl to C conversion factor (Geider *et al.*, 1997), BB apparently only reached or exceeded phytoB in shallow, stratified waters in summer (data not shown) remarkably coincident with a general increase in the apparent bottom-up control (see below), and sporadically at specific depths below the euphotic zone, agreeing with previous reports (Calvo-Díaz and Morán, 2006). The ratio of BB to phytoB showed a strong seasonal pattern mostly caused by changes in phytoB (CV of 131% vs. 49% for BB), with minima (0.03) in March and maxima (3.6) in July. However, except in late winter and spring, values were higher than the 0.2 ratio suggested for coastal and eutrophic zones (Ducklow and Carlson, 1992).

BP (Fig. 1B) was generally low throughout the annual cycle. The three stations displayed somewhat different temporal patterns. Higher volumetric values were found during all summer at St 1, whereas relative peaks were found in March and throughout autumn at St 2 and St 3. Overall, BP followed the variability in chl *a*, although the secondary peak found in September–October coincided with low chl *a* values. Water-column (0–100 m) integrated BP values ranged from 5.4 to 93 mg C m^{-2} day^{-1}, slightly lower than those found a year later at St 2 (7–139 mg C m^{-2} day^{-1}, Franco-Vidal and Morán, 2011).

Billen *et al.* (Billen *et al.*, 1990) proposed that at or near steady state, the use of DOM (dissolved organic matter) by bacteria matches substrate supply rate, which in turn should be equivalent to LIR or BP. This was based on the fact that a strong correlation between BP/LIR and abundance/BB should be good evidence for bottom-up control and the slope could be used as an index of the strength of the control. Briefly, a slope >0.6 would indicate strong bottom-up control, between 0.4 and 0.6 moderate control, between 0.2 and 0.4 weak control and the lack of control would be represented by slopes ≤0.2 or non-significant correlation (Ducklow, 1992).

The effect of substrate supply on BB was analyzed monthly during 2006 at the three stations (Fig. 2). In addition, we calculated the same index during 2007 at St 2 (Franco-Vidal and Morán, 2011) to test for the consistency in the seasonal pattern observed during the previous year (Fig. 2). We also evaluated the role of temperature as the slope of simple linear regression of temperature on BP (natural log-transformed). Months with a temperature range lower than 0.1°C (February 2006 and 2007, December 2007) were not included in the analysis.

Weak-to-strong bottom-up control was consistently detected in summer–early autumn, indicating that resource limitation of bacteria was maximal from July to October (Fig. 2). Conversely, temperature was apparently the main factor controlling bacteria in winter and spring (Fig. 2), coincident with a very consistent lack of effect of substrate supply on BB (slopes ≤0.2 from January to April–May). This is evidence of excess of DOM for bacterial uptake. Bottom-up control would integrate both organic and inorganic substrates, without any differentiation between autochthonous or allochthonous origin, although the former tends to be stronger in periods of low absolute bottom-up control (i.e. winter and spring, Morán *et al.*, 2010). Remarkably, the increase in the strength of bottom-up control was mirrored by a decrease in the corresponding control by temperature. Figure 3 shows a significantly negative exponential relationship between both modes of control for the 2 years ($y = -0.26\, e^{-3.05x}$, $r^2 = 0.62$, $P < 0.0001$, $n = 19$).

When compared with other studies in different regions of the world ocean, this consistent alternation of the

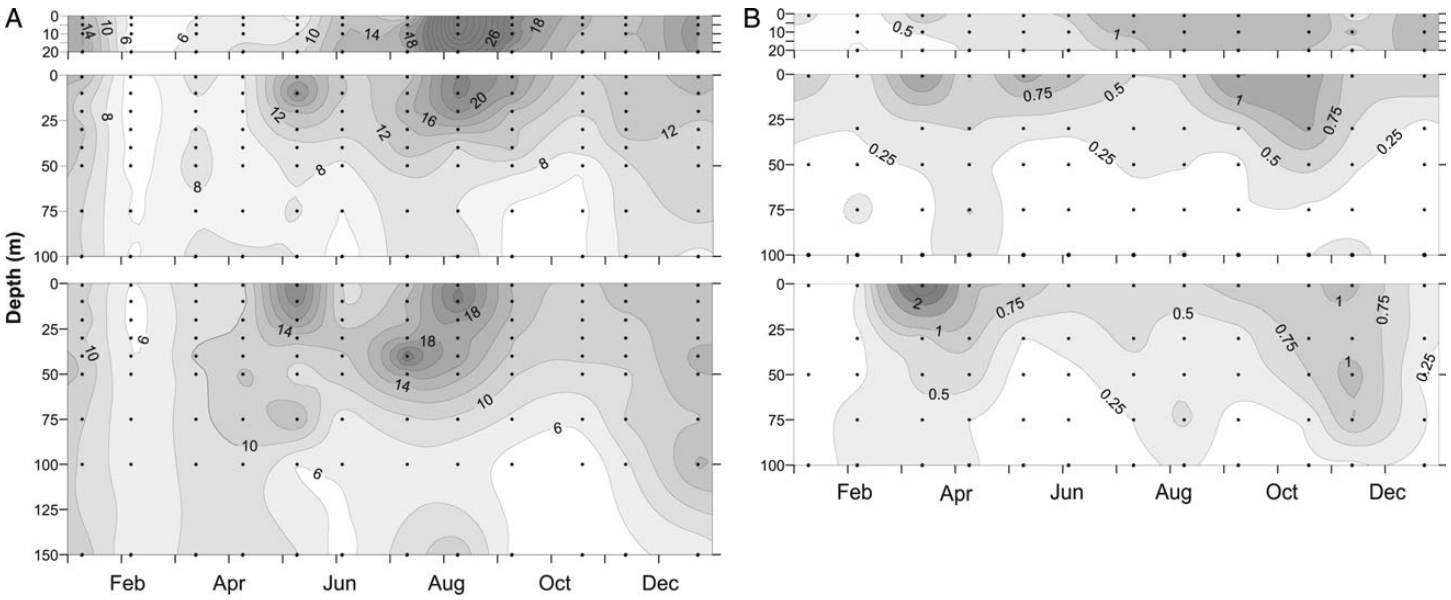

Fig. 1. Vertical distribution of (**A**) bacterial biomass (BB, μg C L^{-1}) and (**B**) bacterial production (BP, μg C L^{-1} day^{-1}) during 2006 in the three sampled stations. Dots mean sampling depths.

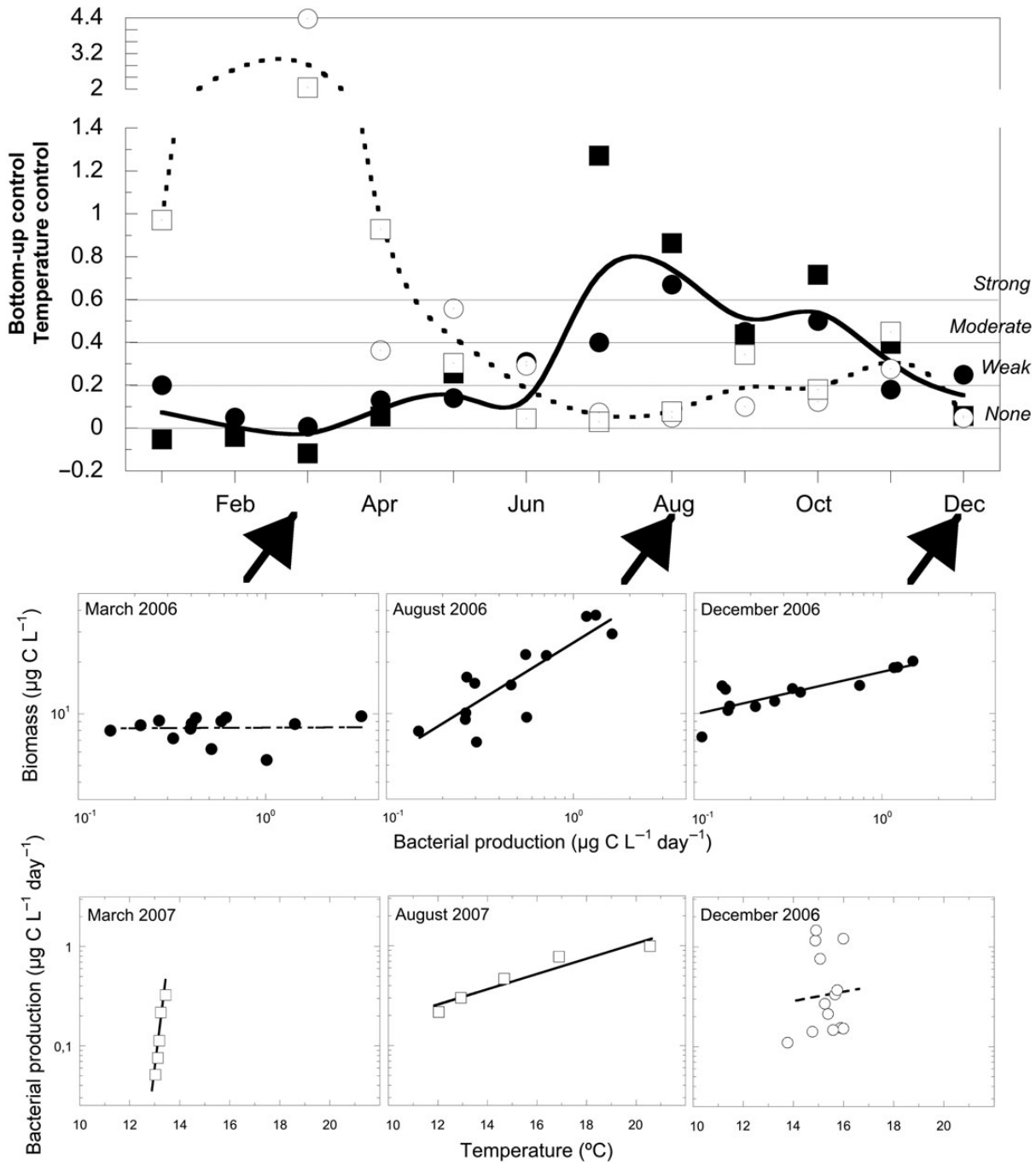

Fig. 2. Monthly variations in the indices of bottom-up control (black symbols) and temperature control (open symbols) for the 2 years sampled (circles for 2006 and squares for 2007). An indication of the strength of bottom-up control according to Ducklow's (Ducklow's, 1992) criterion is also shown. Actual fitted values for selected months are shown as examples. Details of the calculations are given in the text.

relative importance of temperature and substrate supply observed in 2 consecutive years in the Cantabrian sea continental shelf strongly suggest this is a common feature of temperate pelagic ecosystems. The earlier suggestion of a switch between temperature and substrate supply as major bacterial controlling factors was based on a negative relationship very similar to our Fig. 3 in a salt-marsh tidal creek (see Fig. 3 in Shiah and Ducklow, 1995). Later, Shiah *et al.* (Shiah *et al.*, 2003) showed a seasonal shift in the relative importance of temperature and substrate supply as controlling factors in the East China Sea shelf. The dominance of temperature over substrate

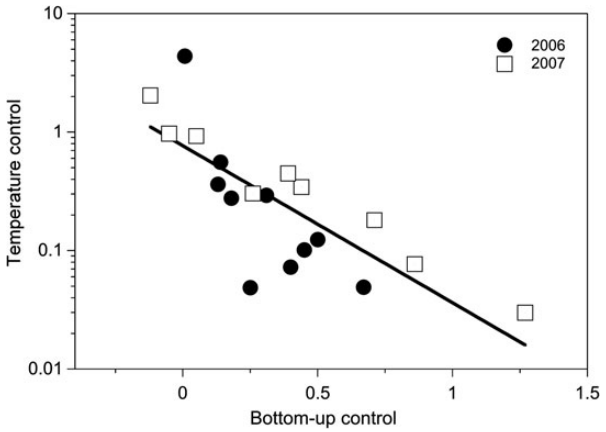

Fig. 3. Relationship between the indices of bottom-up and temperature controls for the 2 years sampled.

regulation could only occur when substrate supply is higher than bacterial demand, as suggested for winter and spring in our study.

Even for the narrow temperature differences found in well-mixed waters (<0.5°C) we would conclude that temperature plays an important role as a bacterial controlling factor during cool, nutrient-sufficient months, where the control of bacterial growth and metabolism by phytoplankton would also be maximum (Morán *et al.*, 2010). However, when higher temperatures allow faster bacterial growth, cells also require greater rates of substrate supply and substrates could become limiting (Shiah and Ducklow, 1994) as found during summer stratification in our study region. Our results demonstrate that the strength of both temperature and substrate supply controls on bacterioplankton changed coherently among seasons, and we suggest that this could be a general feature of bacterial biomass and production dynamics in temperate coastal waters.

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REFERENCES

Billen, G., Servais, P. and Becquevort, S. (1990) Dynamics of bacterioplankton in oligotrophic and eutrophic aquatic environments: bottom-up or top-down control? *Hydrobiologia*, **207**, 37–42.

Calvo-Díaz, A. and Morán, X. A. G. (2006) Seasonal dynamics of picoplankton in shelf waters of the southern Bay of Biscay. *Aquat. Microb. Ecol.*, **42**, 159–174.

Calvo-Díaz, A. and Morán, X. A. G. (2009) Empirical leucine-to-carbon conversion factors for estimating heterotrophic bacterial production: seasonality and predictability in a temperate coastal ecosystem. *Appl. Environ. Microbiol.*, **75**, 3216–3221.

Church, M. J. (2008) Resource control of bacterial dynamics in the sea. In Kirchman, D. L. and Mitchell, R. (eds), *Microbial Ecology of the Oceans*, 2nd edn. Wiley-Liss, New York, pp. 335–382.

Ducklow, H. W. (1992) Factors regulating bottom-up control of bacterial biomass in open ocean plankton communities. *Arch. Hydrobiol. Beih. Ergebn. Limnol.*, **37**, 207–217.

Ducklow, H. W. and Carlson, C. A. (1992) Oceanic bacterial productivity. In Marshall, K. C. (ed.), *Advances in Microbial Ecology*. Plenum, New York, pp. 113–181.

Franco-Vidal, L. and Morán, X. A. G. (2011) Relationships between coastal bacterioplankton growth rates and biomass production: Comparison of leucine and thymidine uptake with single-cell physiological characteristics. *Microbial Ecol.*, **61**, 328–341.

Fuhrman, J. A. (1992) Bacterioplankton roles in cycling of organic matter: the microbial food web. In Falkowski, P. G. and Woodhead, A. D. (eds), *Primary Productivity and Biogeochemical Cycles in the Sea*. Plenum Press, London, pp. 361–383.

Fukuda, R., Ogawa, H., Nagata, T. *et al.* (1998) Direct determination of carbon and nitrogen contents of natural bacterial assemblages in marine environments. *Appl. Environ. Microbiol.*, **64**, 3352–3358.

Geider, R. J., MacIntyre, H. L. and Kana, T. L. (1997) A dynamic model of phytoplankton growth and acclimation: responses of the balanced growth rate and the chlorophyll a:carbon ratio to light, nutrient-limitation and temperature. *Mar. Ecol. Prog. Ser.*, **148**, 187–200.

González, N., Anadón, R. and Viesca, L. (2003) Carbon flux through the microbial community in a temperate sea during summer: role of bacterial metabolism. *Aquat. Microb. Ecol.*, **33**, 117–126.

Morán, X. A. G. and Calvo-Díaz, A. (2009) Single-cell vs. bulk activity properties of coastal bacterioplankton over an annual cycle in a temperate ecosystem. *FEMS Microbiol. Ecol.*, **67**, 43–56.

Morán, X. A. G., Calvo-Díaz, A. and Ducklow, H. (2010) Total and phytoplankton mediated bottom-up control of bacterioplankton change with temperature in NE Atlantic shelf waters. *Aquat. Microb. Ecol.*, **58**, 229–239.

Norland, S. (1993) The relationship between biomass and volume of bacteria. In Kemp, P. F., Sherr, B. F., Sherr, E. B. and Cole, J. J. (eds), *Handboook of Methods in Aquatic Microbial Ecology*. Lewis Publishers, Boca Raton, pp. 303–307.

Shiah, F.-K. and Ducklow, H. W. (1994) Temperature and substrate regulation of bacterial abundance, production and specific growth rate in Chesapeake Bay, USA. *Mar. Ecol. Prog. Ser.*, **103**, 297–308.

Shiah, F.-K. and Ducklow, H. W. (1995) Multiscale variability in bacterioplankton abundance, production and specific growth rate in a temperate salt-marsh creek. *Limnol. Oceanogr.*, **40**, 55–66.

Shiah, F.-K., Gong, G. C. and Chen, C.-C. (2003) Seasonal and spatial variation of bacterial production in the continental shelf of the East China Sea: possible controlling mechanisms and potential roles in carbon cycling. *Deep-Sea Res. Part II*, **50**, 1295–1309.

Shiah, F-.K., Gong, G. C. and Liu, K. K. (1999) Temperature vs. substrate limitation of heterotrophic bacterioplankton production across trophic and temperature gradient in the East China Sea. *Aquat. Microb. Ecol.*, **17**, 247–254.